

# microRNA-378a-5p IS A NOVEL POSITIVE REGULATOR OF MELANOMA PROGRESSION

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Running title: microRNA-378a-5p affects melanoma progression

§This paper is dedicated to the memory of our wonderful colleague, Marianna Desideri, who made a contribution to this project before she passed away. It is a tribute to her enthusiasm and joyful approach to life and work, and to her friendship.

## SUPPLEMENTARY FIGURE LEGENDS

**Figure S1: miR-378a-5p (here abbreviated to miR-378) expression affects the expression of target genes in M14 melanoma cells.** (a) qRT-PCR analysis of STAMBP, SP1, KLF9, FUS-1 and SUFU mRNA expression in M14 cells transiently transfected with mimic miR-378a-5p (mimic miR-378) or mimic miRNA scramble control (mimic Ctrl). The results are reported as fold of reduction in mimic miR-378a-5p (mimic miR-378) transfected cells relative to control ones. Putative target prediction was conducted by miRWalk\* ([http://mirwalk.umm.uni-heidelberg.de/search\\_mirnas/](http://mirwalk.umm.uni-heidelberg.de/search_mirnas/)). The primers used to analyze each gene are listed in Supplementary Table S2. (b) Western blot analysis of STAMBP, SP1, KLF9 and SUFU protein expression in M14 cells transfected with mimic Ctrl or mimic miR-378. Representative images of one out of two independent experiments are reported. HSP72/73 was used as loading and transferring control. (c) qRT-PCR analysis of STAMBP, SP1, KLF9, FUS-1 and SUFU mRNA expression in M14 cells transiently transfected with miR-378a-5p inhibitor (anti-miR-378) or with mimic miRNA scramble inhibitor control (anti-miR-Ctrl). The results are reported as fold induction in anti-miR-378 transfected cells relative to control ones. (a,c) The average  $\pm$  SEM of three independent experiments performed in triplicate is reported. Statistical analysis was performed applying T-test,  $*p<0.05$ ,  $**p<0.01$ ,  $***p<0.001$ . (d) Luciferase assay showing miR-378a-5p binding to 3'UTR region of STAMBP mRNA. Expression vectors carrying a luciferase reporter followed by the 3'UTR regions of STAMBP in their wild-type (wt, black bars) or mutated (mut, gray bars) forms, in the miR-378 complementary sequence were transfected in A375 cells in the presence of mimic Ctrl or mimic miR-378. Normalized luciferase activities of mimic miR-378 transfected cells respect to control were reported. (a,c,d) Statistical analysis was performed applying T-test,  $*p<0.05$ .

**Figure S2. miR-378a-5p (here abbreviated to miR-378) expression does not affect *in vitro* cell proliferation and colony formation ability of M14 cells.** Evaluation of (a) *in vitro* proliferation and (b) colony formation ability of M14 cells transfected with mimic miR-378a-5p (mimic miR-378), or miR-378a-5p inhibitor (anti-miR-378) or with scramble controls (mimic Ctrl, anti-miR-Ctrl).

**Figure S3: miR-378a-5p (here abbreviated to miR-378) expression increases *in vitro* migration and invasion of melanoma cells.** Representative images of *in vitro* cell (a) migration of M14, A375 and SBCL1 cells and (b) invasion of M14 and A375 cells transiently transfected with mimic miRNA scramble control (mimic Ctrl) or mimic miR-378a-5p (mimic miR-378). For *in vitro* cell migration of SBCL1 cells, the quantification of migrated cells/field is reported in the bar chart as

fold respect to cells transfected with mimic control. Statistical analysis was performed applying T-test, \*\*\* $p < 0.001$ .

**Figure S4: miR-378a-5p (here abbreviated to miR-378) expression increases *in vitro* vasculogenic mimicry of melanoma cells with a VEGF-dependent mechanism.** Representative images of *in vitro* capillary-like structure formation of (a) M14 and (b) SBCL1 cells transiently transfected with mimic miRNA scramble control (mimic Ctrl) or with mimic miR-378a-5p (mimic miR378) in absence or presence of VEGF or IL-8 (anti-VEGF or anti-IL-8) neutralizing antibodies. For *in vitro* vasculogenic mimicry of SBCL1 cells, the quantification of intersection points/field is reported in the bar chart. Statistical analysis was performed applying T-test, \*\*\* $p < 0.001$ .

**Figure S5: miR-378a-5p (here abbreviated to miR-378) expression increases *in vitro* cell migration and invasion of melanoma cells with a uPAR-dependent mechanism.**

Representative images of *in vitro* cell (a) migration and (b) invasion of M14 cells transiently transfected with mimic miR-378a-5p (mimic miR-378) incubated with oligonucleotides directed against uPAR (si-uPAR), or with M25 peptide. Scramble siRNA (si-Ctrl) and scramble peptide (peptide-Ctrl) were used as control.

**Figure S6: miR-378a-5p (here abbreviated to miR-378) expression increases *in vitro* vasculogenic mimicry of melanoma cells with a uPAR-dependent mechanism.** Representative images of *in vitro* capillary-like structure formation of M14 cells transiently transfected with mimic miRNA scramble control (mimic miR-Ctrl) or mimic miR-378a-5p (mimic miR-378) incubated with oligonucleotides directed against uPAR (si-uPAR) or scramble siRNA (si-Ctrl).

**Figure S7. Schematic figure depicting the identified players in regulating miR-378a-5p expression and its target genes reported in cancer and biological effects observed in melanoma.** For target genes, different colors indicate the level of evidence. Blue: changes in mRNA and protein expression after miRNA transfection; Red: changes in mRNA and protein expression after miRNA transfection and proof of direct interaction by reporter assay and site-specific mutagenesis; Green: changes in mRNA and protein expression after miRNA transfection, proof of direct interaction by reporter assay and site-specific mutagenesis and negative correlation of expression in patients. The players and the target genes investigated in the present paper are indicated in bold type.